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A SPECIFIC ABNORMALITY ASSOCIATED WITH A VARIETY OF GENOTYPES

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We have found recently a hitherto undescribed hereditary syndrome of abnormalities in the house mouse, in which a similar set of developmental changes in the gut and urogenital system appears as the expression of any one of about a dozen different genotypes, comprising seven different mutations with marked effects on the axial skeleton and tail. The results of a preliminary study suggest that the abnormality arises whenever some process in early development is sufficiently deflected from its normal path and that the different mutations affect this process in different degrees. It can be inferred from the genetical study that the process involves an inductive relationship by which the normal development of the posterior gut and urogenital system is connected with the development of the axial system.

An account of the morphology of the syndrome and of a detailed analysis of its inheritance and expression in some of the genotypes is being published elsewhere. The present note records the occurrence of the abnormality as a common type of response to a number of mutant changes.

The syndrome was first noted in one family of tailless mice in which certain offspring which were alive at birth lacked both anal and genital apertures and died shortly after birth. Further study of such abnormal animals from this and other stocks showed that the essential feature of this abnormality in all stocks is the simultaneous failure of development of the posterior parts of the gut and of the urinary duct, often resulting in an abnormal condition in which colon and urethra remain connected, forming a cloaca which, however, has no external opening. Nearly always the rectum is absent or vestigial and the anus fails to form. In some cases, kidneys, ureters, genital ducts and bladder are missing or abnormal but these are not constant parts of the basic syndrome and the frequency of these abnormalities varies in different genotypes. The syndrome is

diagnosed at birth by the imperforate anus, absence or underdevelopment of the genital papilla and complete failure of urinary excretion. It is probably the latter feature which brings about the early death of animals with this syndrome. This abnormality has been found regularly in the following genotypes at birth:

1. *Sd Sd*. *Sd* is a dominant mutation producing a short tail and kidney defects in heterozygotes; homozygotes *Sd Sd* always show the full syndrome of imperforate anus and true cloaca without excretory duct and in addition are tailless and generally have no kidneys (cf. Gluecksohn-Schoenheimer[1]).

2. Tt^0 *uu*. Tt^0 is tailless (cf. Dunn[2] for review of effects of *T* and related tail mutations); *u* is a new recessive which in combination with Tt^0 produces the imperforate anus and urogenital syndrome in all or nearly all *uu* animals.

T+ *uu*—short-tailed animals which show the syndrome in a large proportion but not in all homozygotes.

++ *uu*—animals with short contorted tails, weak, sterile and stunted; but showing the syndrome in a small proportion (about 10 per cent) of homozygotes.

3. *Ki T*/++; *Ki* (Kink tail) is a mutation closely linked with *T*, and producing in combination with *T* a shortened kinky tail or complete taillessness (Dunn and Caspari[3]). The syndrome has appeared in about half of the animals of this genotype, often accompanying an abnormality of the spinal column, spina bifida aperta, in the sacral region. It appears also in animals of genotype *Ki*/*T* but its frequency in this genotype has not been established.

4. *Ki T*/+ t^n; this genotype is always tailless, frequently shows spina bifida aperta, and more than half of the newborn animals have the imperforate syndrome and die shortly after birth.

5. *Ki T*/++; *Sd*/+; tailless, often with syndrome and with absence or abnormality of kidneys more severe than is found in *Sd*/+.

6. *Ki*/+; *Sd*/+; tailless; occasionally like 5.

7. *Fu*/*Fu*; *Fu* is a tail mutation closely resembling *Ki*. It acts as a semilethal, some *Fu Fu* being viable. *Fu* is closely linked with *Ki* and *T* (Dunn and Caspari[3]). In nearly all *Fu Fu* animals at birth (25 out of 26 dissected) the kidneys are missing or reduced; while 9 out of 26 showed the imperforate syndrome.

8. $Fu/t^0 \times Fu/t^0$. The offspring of this cross are *Fu*/*Fu* or Fu/t^0 since t^0 is lethal and prevents recombination in this area (Dunn and Caspari, in press). Many of the offspring show the same conditions as described in 7 above.

9. *Fu*/+; *Sd*/+. This combination frequently shows the imperforate syndrome and nearly always has kidney defects more severe than

those associated with *Sd* alone. It appears that *Fu* and *Sd* show some cumulative effect in increasing the extent of urogenital abnormality.

10–11. In litters containing the genotypes $\frac{Fu\ +}{+\ T}$ and $\frac{Ki\ T\ +}{++Fu}$, animals with the syndrome and suspected to be of the above genotypes have been found. Because such animals die shortly after birth it has been impossible to verify the diagnosis of genotype.

In general, it can be seen that the same syndrome or parts of it tend to appear when *u*, *Sd*, *Ki* and *Fu*, are present in combination with each other or with *T*, t^0 or t^1. It does not appear when only the latter mutations are present. The frequency with which the syndrome appears in the different genotypes is variable and probably depends in part on factors, other than those named, in which the animals differ, since it has not yet been possible to prepare isogenic stocks. For example, when the *Ki T* chromosome was derived from one male about half of the offspring which received it showed the syndrome, while of those receiving it from other parents, a much smaller proportion were abnormal. In spite of this variability, it is evident that animals homozygous for *Sd Sd* always show the full syndrome, while those with *uu* Tt^0 nearly always show it. One other homozygous combination, *Fu Fu*, is sometimes viable until after birth, and although it shows the imperforate condition in only about a third of the newborns, all of them show related effects on the kidneys. Homozygosity for one of the three factors *Sd*, *u* or *Fu* thus produces a high probability that urogenital development will be abnormal. The other mutations involved, *Ki T*, t^0 and t^1, are all lethal before birth when homozygous and their effects on producing the syndrome can only be observed in heterozygous combination. It is noteworthy that of the many simple heterozygotes observed, *Ki*/+, *T*/+, t^0/+, t^1/+, *Fu*/+, *u*/+ and *Sd*/+ none has been found with this abnormality. On the other hand, it is often found in animals heterozygous for *two or more* of these mutations, *e.g.*, *Ki T*; *Ki T*/t^0 or t^1; *Fu*/t^0 or t^1; *Fu*/*T*; *Fu*/+*Sd*/+; *Ki*/+*Sd*/+, etc., indicating that one dose of each of these is able to increase the effect of one dose of any other one, in a manner similar to the interaction of two doses of the same allele in the viable homozygotes such as *Sd Sd*. The frequency of abnormal animals, however, appears to be lower among heterozygous combinations than among homozygotes.

These facts may be explained on the following hypothesis. Each of the mutations concerned has a quantitative effect on some process concerned with development of the posterior spine tending to cause it to fall below its normal level. This produces abnormalities or absence of tail vertebrae in all combinations of these mutations, and abnormalities in the sacral region at least in most combinations. (Spina bifida aperta is regularly found in *Sd Sd*, often in *Ki T*/t^0 or t^1, *Ki*/*T*, *Ki T*/*Fu*, *Fu*/t^0 or t^1,

Fu/Fu.) When the posterior axial region is sufficiently defective, it fails to provide the normal inductive stimuli which regulate the development of the posterior gut and urinary ducts, and particularly the separation of the urogenital sinus from the rectum. This assumption is based not on embryological evidence but on the evident connection between abnormalities of the posterior spine and urogenital system.

The hypothesis implies that the mutations studied (*u*, *Sd*, *Ki*, *T*, *Fu*, t^0 or t^1) have some effect in common which underlies the process or processes leading to the abnormal connection between gut and urinary duct. They apparently differ in their effects on certain other processes since each mutant probably has peculiar effects on tail form by which it can be identified. Further study may, of course, reveal differences in the urogenital effects of the different mutants, but the essential features of the syndrome, failure of posterior gut and urinary duct and absence of anus, appear to be the usual type of response of the embryo to anatomical defects brought about by a number of independent mutations.

* The experiments reported were carried out with the aid of a grant from the Josiah Macy, Jr., Foundation of which grateful acknowledgment is made.

[1] Gluecksohn-Schoenheimer, S., *Genetics*, **28**, 341–348 (1943).

[2] Dunn, L. C., *Growth*, **5**, 141–161 (1941).

[3] Dunn, L. C., and Caspari, E., these PROCEEDINGS, **28**, 205–210 (1942).

[4] Reed, S. C., *Genetics*, **22**, 1–13 (1937).

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*CAN SPECIFIC MUTATIONS BE INDUCED BY SEROLOGICAL METHODS?**

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It has been suggested by Haldane (1935) and by Irwin (1939) that many of the erythrocyte antigens of birds and mammals are rather direct gene products. The reason for this view is that (if we except a portion of the antigens present in species-hybrids of doves) there is a one-to-one correspondence between the presence of specific single genes and specific antigens (see summaries for man by Boyd, 1939, and Wiener, 1935, for rabbits by Castle and Keeler, 1933, for fowls by Todd, 1935, for doves and pigeons by Irwin, 1939). In these cases there is—with the exception noted—no evidence for gene interaction, either between allelomorphs or between genes at different loci.

The same conclusions may be applied to the genes and antigens responsible for the specificity of transplantation compatibility in mammals. The results of Loeb and Wright (1927) with guinea pigs show that transplantation is successful only if all the specific genes of the donor are present in the host. This evidently means that the host forms antibodies to any antigen present in the donor tissue, unless the same antigen is present in the host also. The genetic results mean further that these antigens are numerous, and that here also there is no gene interaction. Similar conclusions may be drawn from the experiments on tumor transplantation in mice.

As pointed out by Haldane (loc. cit.), this conclusion suggests that there may be some common configuration in the molecular structure of an antigen and that of the gene responsible for it. If this common configuration concerns that part of the antigen that is responsible for its specificity—and such a relation is implied in the suggestion—there are certain further possibilities to be investigated.

As is well known (see discussion by Landsteiner, 1936), there are two distinct properties of an antigen—that of inducing the formation of a specific antibody, and that of reacting with the specific antibody. These two properties evidently depend on the same specific configuration; but for the first it is usually (probably always) necessary that the specific configuration be present in or chemically attached to a protein, while for the reaction with the antibody such an association is not necessary.

These considerations lead to the supposition that, if a particular gene is responsible for the formation of a given antigen, then there is a possibility that the antibodies induced by this antigen may react with the gene. If this possibility exists there is a series of consequences that are of interest to the geneticist.

One such consequence is that it becomes possible to interpret the old experiments of Guyer and Smith (1920, 1924) with rabbit lenses. As is well known, the lens protein has specific antigenic properties. Guyer and Smith reported that they immunized fowls to rabbit lenses, and injected the immune sera into rabbits. There resulted lens defects in the offspring, and these defects were inherited, both through females and through males. These experiments have been criticized, and Finlay (1924), Huxley and Carr-Saunders (1924) and Ibsen and Bushnell (1934) have failed in attempts to confirm them. I am, however, informed by Professor R. R. Hyde that his experiments (still in progress) have given confirmatory evidence. On the basis here suggested, the mechanism of such a result is clear: The lens protein does not ordinarily get into the circulation, so the animal neither forms antibodies to it nor binds them when immune serum is injected. These antibodies are, therefore, free in the circulation in the injected rabbits, and some of them combine with the specific genes (in the

germ cells) that are responsible for lens antigen—presumably inactivating them. If the results can be confirmed, and if this interpretation is correct, we have here a method for the induction of a specific mutation.

Guyer and Smith interpreted their results as demonstrating the inheritance of an acquired character. On the interpretation here suggested, it becomes a matter of terminology whether they are properly to be so described. It does appear, however, that other cases cited in support of the Lamarckian view should be reëxamined with the gene-antigen-antibody possibility in mind. A casual survey of the literature leads me to suppose that the most likely additional example is that reported years ago by Brown-Sequard. In this case the interpretation might be that guinea pigs are capable of producing antibodies to their own nervous tissue when it is injured.

* This manuscript was written in 1940, and was submitted to Dr. Hyde, whose experiments are referred to as forming much of the basis for the conclusions. Dr. Hyde gave his permission for the reference to his work; but I felt that he would prefer to wait until he could carry out more experiments, and I therefore did not publish. Two factors now influence me to publish the note: Dr. Hyde's death in 1943, without his having published his results; and the results of Dr. Emerson, described in the accompanying paper. The note stands as written in 1940, though a few minor changes might now be desirable. For more recent references, see the paper of Dr. Emerson.

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THE INDUCTION OF MUTATIONS BY ANTIBODIES*

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In most biological syntheses each step is controlled by a different gene. This conclusion was suggested by such combined genetic and biochemical studies as those on the anthocyanin pigments[1] from which it appeared that a particular gene regulates the oxidation (or methylation, or the nature of the glucosides) at particular carbon atoms of the anthocyanin molecules. Studies of the eye pigments of Drosophila[2] and the hair pigments of guinea pigs[3] each indicate that the different steps in a particular synthesis are controlled by different genes. Almost overwhelming support for the conclusion is coming from studies of "biochemical" mutations in Neurospora.[4]

Studies of inherited antigenic differences in many species[5] agree in showing that the immunological specificity of an individual antigen is determined by a single gene, as if the development of antigenic specificity represents but a single step in the synthesis of the antigen. The synthesis of genes may represent an analogous instance. "The most fundamental —in fact, the unique and distinctive—characteristic of a gene is . . . the fact that, in its protoplasmic setting, it produces a copy of itself, next to itself, and that when its own pattern becomes changed, the copy it now builds is true to its new self."[6] In the terminology of this report: the specificity of a gene is determined solely by a gene of identical specificity to that synthesized.

The possibility that a gene impresses a copy of its own antigenic specificity on the antigen it regulates can, under sufficiently favorable circumstances, be tested experimentally. Antibodies to such cellular antigens should combine with the genes whose antigenic structure is the same as that of the immunizing antigen. There is no certainty that the gene-antibody reaction can be recognized by the usual serological techniques. There is the chance, however, that if antibodies combine with genes at the time they are "producing copies of themselves," the presence of the antibody molecule would so alter the surface of the gene that the copy produced would have a changed structure. Such alteration in genic structure should, on the basis of this reasoning, result in some change in the physiological or developmental step regulated by the gene in question, and the antibody-induced change should be recognizable as a mutation, as pointed out by Sturtevant in the preceding paper.[7]

While the series of events just postulated presents numerous conditions to be met in an experimental attack, there are certain recorded cases which

appear to fit this interpretation. Of these the only one in which antibodies to a relatively pure antigen have induced changes of proved heritable nature is that reported by Guyer and Smith and discussed by Sturtevant.[7] The instances of apparent induction of mutations in bacteria by specific antisera, while suggesting the same interpretation, do not fulfil all conditions since the absence of sexual reproduction in these organisms makes the genetic proof of mutation impossible. Probably the most thoroughly worked out example of changes following treatment with antisera is the smooth to rough transformation in pneumococcus.[8] The smooth, or virulent, form produces a polysaccharide envelope (chemically and immunologically specific to the type) which is absent in the rough form. Changes from smooth to rough rarely occur spontaneously, but are abundantly produced by growth in antiserum to the specific type. While, from the published accounts, it seems probable that the loss of the capacity to synthesize the polysaccharide is directly induced by antibodies to the polysaccharide, it is still impossible to rule out the alternative interpretation that the antibodies act as a powerful selective agent favoring those rough cells which occasionally arise spontaneously.

The ascomycete, *Neurospora crassa*, was selected for the experiments to be reported because of its favorable genetic and cultural characteristics.[9] The general plan of the experiments was to treat the fungus with antisera (developed in rabbits to mycelial extracts or culture filtrates), isolate strains descended from single nuclei (obtained by crossing to the opposite sex and selecting ascospores), and to test the isolates for deficiencies in growth on different carbohydrates.[10] The results of the experiments are summarized in the accompanying table.

To test the genetic homogeneity of the strains used (Beadle's 1A and 25a) conidia of one sex were dusted over cultures of the other sex in which properithecia had already developed. Since the nuclei from one conidium do not ordinarily take part in fertilization in more than one perithecium,[11] it is possible to sample genes from a large number of nuclei of the conidial parent by testing one isolate from each perithecium. The untreated controls listed in the table were handled in this way. Whenever conidia were treated with antisera the isolates were obtained in the same way, but in those instances in which the mycelial stage was treated the method was modified, and there is the possibility that more than one isolate was descended from a single treated nucleus.

The data indicate a much greater variability in the treated material than in untreated controls. No statistical analysis is attempted because the tests for variants were not strictly comparable from one experiment to another. In any one experiment, however, the controls were tested at the same time and in the same way as the treated isolates, and the differences are consistent throughout.

Of the variants listed, eleven have so far been subjected to genetic test, and each of these has differed from the wild-type parents by a single gene. Evidence from genetic tests and from tests of heterocaryons[12] indicates that four variants (Nos. 1077, 1083, 1092 and 1095) from one treatment represent different alleles of a gene by which the parent strains (1A and 25a) differ from another pair of wild-type strains (Beadle's 4A and 12a). At least three other variants represent mutations at three other loci.

MATERIAL TREATED	TREATMENT	IMMUNIZING ANTIGEN	CROSSED TO	TOTAL ISOLATES	VARIANTS NO.	VARIANTS IDENTIFICATION
Mycelium of 25a	50% antiserum, 27 hrs. at 25°	Mycelial extract	Untreated 1A	16	1	No. 859
	50% antiserum, 27 hrs. at 25°	Culture filtrate	Untreated 1A	21	0	...
	50% normal serum, 27 hrs. at 25°	None	Untreated 1A	12	0	(Control)
Conidia of 1A	100% antiserum, 3 hrs. at 25°	Culture filtrate	Untreated 25a	52	4	Nos. 1077, 1083, 1092 1095
	None	None	Untreated 25a	63	0	(Control)
Mycelium of 1A	100% antiserum, 48 hrs. at 25°	Culture filtrate	25a, same treatment as 1A	128	1	No. 1874
	Same, but two 2-day transfers	Culture filtrate	25a, same treatment as 1A	71	2	Nos. 1905, 2606
	Same, but four 2-day transfers	Culture filtrate	25a, same treatment as 1A	210	2	Nos. 2195, 2679
	Same, but five 2-day transfers	Culture filtrate	Untreated 25a	94	1	No. 2012
Conidia of 1A	None	None	Untreated 25a	170	0	(Coptrol)
Conidia of 25a	100% antiserum overnight at 2°	Starch adsorbed enzymes	Untreated 1A	13	2	Nos. 5131, 5168
	100% antiserum overnight at 2°	Starch adsorbed enzymes	Untreated 1A	30	4	Nos. 5175, 5177, 5269, 5294
	100% antiserum overnight at 2°	Amm. sulphate preciptated culture filt.	Untreated 1A	31	3	Nos. 5139 5191, 5201
	100% antiserum overnight at 2°	Amm. sulphate precipitated culture filt.	Untreated 1A	29	5	Nos. 5007, 5008, 5031, 5204, 5211
	None	None	Untreated 1A	31	0	(Control)
			Total treated	695	25	
			Controls	276	0	

With the exception of two morphological mutations, all variants so far obtained differ from the wild type by deficient growth [13] when cultured at standard temperatures and on carbohydrate substrates normally utilized by the wild type. None has yet been obtained which fails to use a particular carbon source under all conditions.

The data just presented indicate that antibodies (or possibly some other serum constituent whose rôle is unsuspected) influence the fre-

quency of recoverable mutations. The indications are that the mutations are induced rather than selected by the treatment, since prolonged treatment of mycelium (during which considerable growth occurs) seems to be less effective than a relatively short treatment of conidia (where few nuclear divisions can have occurred). This difference was especially noticeable when conidia were treated at low temperatures which tend to favor more complete antigen-antibody reactions (e.g., in the precipitian and complement-fixation tests). The apparent deficiency of variants following prolonged growth in antiserum can be interpreted either as a result of selection against the mutated nuclei, or on the assumption that nuclei already affected are more likely to be further affected by antibodies, resulting in inviability.

While the immunizing solutions were known to contain many carbohydrases, the antibodies produced have not been identified in terms of specific antigens.[14] On the present interpretation, the four members of an allelic series may have arisen in response to one sort of antibody, the three independent genes to three other sorts.

The material seems well suited to the problem under discussion, but there are certain refinements to be adopted. Fertile wild-type stocks which lack the growth deficiencies of 1A and 25a, and which give as little variation as possible when intercrossed are being developed. Further, in order to observe a direct relationship between specific genes and antibodies it will be necessary to make use of antigens which can be isolated in relatively pure form. It may be essential to select antigens which are not in themselves indispensable to the organism, otherwise viable mutations are not to be expected.

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[1] References in Scott-Moncrieff, R., *Jour. Genetics*, **32**, 117–170 (1936), and Lawrence, W. J. C., and Price, J. R., *Biol. Rev.*, **15**, 35–58 (1940).

[2] References in Beadle, G. W., and Tatum, E. L., *Amer. Nat.*, **75**, 107–116 (1941), and Ephrussi, B., *Quart. Rev. Biol.*, **17**, 327–338 (1942).

[3] References in Wright, S., *Biol. Symposia*, **4**, 337–355 (1942).

[4] Beadle, G. W., and Tatum, E. L., *Proc. Nat. Acad. Sci.*, **27**, 499–506 (1941); Tatum, E. L., and Beadle, G. W., *Growth, Fourth Growth Symposium*, **6**, 27–35 (1942), etc.

[5] For example, the heritable erythrocyte antigens of man (Landsteiner and others), of fowls (Todd), of rabbits (Castle and Keeler), see references in Wiener, S., *Blood Groups and Blood Tranfusion*, 2nd ed., Charles C Thomas, Springfield, Ill., 1939; references to the Rh-factor in man in Wiener, A. S., and Landsteiner, K., *Proc. Soc. Exp. Biol. Med.*, **53**, 167–170 (1943); erythrocyte antigens of doves, Irwin, M. R., *Genetics*, **24**, 707–721 (1939); of cattle, Ferguson, L. C., Stormont, C., and Irwin, M. R., *Jour. Immunol.*, **44**, 147–164 (1942); the serum protein antigens of doves, Irwin, M. R., and Cumley, R. W., *Genetics*, **27**, 228–237 (1942).

[6] Quoted from Muller, H. J., *Cold Spring Harbor Symposia on Quantitative Biology*, **9**, 290–308 (1941).

[7] Sturtevant, A. H., the preceding paper in these PROCEEDINGS.

[8] Reimann, H. A., *Jour. Exp. Med.*, **41**, 587–600 (1925), has references to earlier literature; Felton, L. D., *Ibid.*, **56**, 13–25 (1932); Paul, J. R., *Jour. Bact.*, **28**, 46–66 (1934). See also discussions in Heidelberger, M., *Amer. Nat.*, **77**, 193–198 (1943), and Avery, O. T., McLeod, C. M., and McCarty, M., *Jour. Exp. Med.*, **79**, 137–158 (1943). A different interpretation of the transformations in pneumococcus is given by Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **29**, 338–343 (1943).

[9] References in Lindegren, C. C., *Iowa State Col. Jour. Sci.*, **16**, 271–290 (1942), and Beadle and Tatum (1941), loc. cit., footnote 4.

[10] Tests were usually made on sucrose, cellobiose, maltose, starch and α-amylodextrin, on which the wild type grew "normally." The method is essentially similar to that described by Beadle and Tatum (1941), loc. cit., footnote 4.

[11] It has been shown by Backus, M. P., *Bull. Torrey Bot. Club*, **66**, 63–76 (1939), that conidia of *Neurospora sitophila* do not germinate on agar already supporting mycelial growth, but trichogynes from the properithecia attach themselves to such conidia and withdraw the cellular contents.

[12] The method of testing for allelism with heterocaryons is described by Beadle, G. W., and Coonradt, V. L., *Genetics*, **29**, 291–308 (1944).

[13] Growth characteristics were determined by the "tube method" described by Ryan, F. J., Beadle, G. W., and Tatum, E. L., *Amer. Jour. Bot.*, **30**, 784–799 (1943).

[14] Precipitin titres were too low to permit the utilization of an absorption technique.

A RECONSIDERATION OF THE MECHANISM OF POSITION EFFECT

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The phenomenon of position effect has come in recent years to occupy a central position among problems facing the geneticist, in spite of the restriction of well-established cases to one species. This is, no doubt, due partly to the fact that position effect now clearly appears to be much more frequent in Drosophila than originally suspected; but even more to its possible bearing on the problem of the degree of discreteness of the hereditary material. The real significance of position effect in this connection will depend, of course, on the nature of the mechanism which will be found to be responsible for its occurrence; and that is why the clarification of this mechanism appears so urgent.

Speculations concerning the mechanism of position effect have, on the whole, been surprisingly timid. With the exception of the two papers by Muller,[1] and Offermann,[2] especially devoted to the theoretical discussion of this problem and of Schultz's papers[3, 4] on variegation, the literature on

position effect, particularly in more recent years, contains very little on the subject, and is seemingly more concerned with providing an exhaustive list of possible explanations than with elaborating one of the interpretations to a point where it becomes capable of experimental verification. As will clearly appear below, the reasons for this reticence are many. Yet it seems useful at this stage to reconsider the various possibilities of explanation of position effect and to compare their relative probabilities after putting them in a somewhat more precise, although speculative, form.

We may first put aside some early interpretations according to which the frequent modification of the action of genes located in the neighborhood of chromosomal breaks is due to mutation of one or more loci or to destruction of genes induced at the time of breakage (Bridges,[5] Muller and Altenburg,[6] Muller[7]). Such interpretations, which really are negations of position effect as such, have since been radically disproved in a series of cases by the demonstrated reversibility of position effect. The possibility remains that some of the changes often designated as position effects (e.g., *Curly*, *Dichaete*, etc.) may really be covered by the above interpretation, and were this demonstrable they would thus be excluded from the category of position effects.

All other and more recently formulated hypotheses as to the mechanism of position effect can be said to belong to one of two essentially different types of interpretation to which we may refer as the kinetic and the structural hypotheses.[8]

Interpretations of the first (kinetic) type can be derived from Sturtevant's remark in his very first paper on position effect that the "influence of the relative position of genes on their effectiveness in development may be interpreted in terms of diffusion and localized regions of activity in the cells."[9] The papers of Offermann[2] and Muller[1] referred to above contain rather detailed discussions of position effect "in terms of diffusion," the argument running somewhat as follows. Genes undoubtedly produce their effects on the phenotype by forming specific substances. These substances must gradually diffuse into the protoplasm, and their distribution must follow a gradient of concentration with a maximum concentration in the immediate neighborhood of their centers of production. Between the moment of their production under the control of the genes concerned and their final action responsible for the phenotypic effect, these substances probably undergo numerous changes, due to interaction with the surrounding protoplasm and with substances involved in other gene controlled reaction chains. If some of the interactions involve the immediate products of the activity of two different genes, their outcome may depend on the distance separating the two genes from which the two interacting products originate.

A slightly different variant of the same scheme is mentioned by Waddington:[10] position effect is pictured here as the result of competition of two genes for the same substrate. It is the distribution of the substrate, not that of the resulting products, which becomes decisive in this case and which follows a gradient in a direction opposite to that described above. Again the occurrence of position effect is assumed to depend on the distance between the "spheres of influence" of the genes concerned. This particular variant of the interpretation is quoted here only because, in this form, it brings us very close to a picture of gene action at present used by Stern, *et al.*, in an interpretation, to be discussed below, of the effects of combinations of *cubitus interruptus* alleles and of position effects at this locus.

Contrary to this first general type of interpretation, the characteristic of which obviously is that it tends to explain position effect on the basis of interactions between gene *products*, or competition between genes for a common substrate, the "structural hypotheses"[1, 11] postulate reversible modifications of the genes themselves or of their structural interrelations. One of the simplest interpretations of this type consists in assuming that chemical bonds unite neighboring genes into integrated, larger units, and that within such a unit, as within any large molecule, changes produced in one of its parts will affect the properties of the whole. On this view position effects are comparable to the steric effects familiar to the organic chemist.

It must be noticed, first of all, that without further understanding of mutations and chromosomal breaks, no sharp line can be drawn between changes of the sort discussed and mutations. As stated above, one can still question whether all changes which have arisen concomitantly with chromosomal breaks and are now classified as position effects really belong to a single class of changes and one may not exclude the possibility suggested by Dobzhansky[11] that "some changes in gene position may induce reversible, and other changes irreversible position effects."

It must be pointed out now that the difficulties of the structural hypothesis in this form have been from the very first so fully realized by its author[1] that he definitely gave preference to what we have called the kinetic hypothesis, as shown by the title of his paper "The position effect as evidence of the localization of the immediate products of gene activity." The following quotation gives Muller's own presentation of the case and, in the last sentence, a possible way out of the difficulty: "A little consideration, however, shows such an interpretation of the 'position effect' of genes to be very implausible, since direct chemical influences of the type in question could hardly extend over distances so vast, from the chemical standpoint, as are those here in question. Certainly the connections between the genes, even if chemical, are of a different nature (allowing of crossing over) from those within the genes, and must allow of the relative chemical independence of the latter: the individual genes, moreover, are very long,

from the chemical standpoint, or else lie far apart from one another."... "That, under these circumstances, the direct chemical effect of the substitution of one gene for another should extend, not only to the adjacent gene, so as to affect the latter's function, but even through the latter to one or more genes beyond, is hardly conceivable, unless we postulate some hitherto unfamiliar principle of chemistry, such as might be involved in those forces which are responsible for the synaptic attraction between like genes. The latter are so unusual and act over such relatively great distances that the possibility is not excluded that the same forces which, when acting between genes of identical conformation, cause a harmonious attraction, might, when acting between unlike genes, set up differential stresses on their different parts and so cause distortions in their conformation and consequent changes in their chemical properties."[1] In the latest of Muller's writings[12] on this subject there is rather stronger emphasis on the latter possibility as well as on the striking coincidence of both position effects and somatic pairing in Diptera.

Since the above quoted lines were written the distances over which position effects may extend have been found to be much greater than those implied in Muller's statement. In fact, they are so great in some cases (Demerec,[13]) that, in addition to further emphasizing the impossibility of an explanation of position effect on the basis of steric effects *sensu stricto*, they moreover lead one to question the validity of the kinetic interpretation. The question is: What distance is covered by the assumed gradients of concentration around the genes and how do these distances compare with the distances over which position effects are known to extend? If we reason as above in terms of a consumed substrate, our problem, as suggested by Delbrück in a personal discussion,[14] becomes somewhat similar to that of the adsorption of phage on the surface of bacteria. The latter problem has been discussed by Delbrück[15] from whose paper we quote: "Let us calculate the average density distribution around a resting adsorbing sphere of radius a (bacterium) suspended in a medium that contains initially a uniform density of particles which may stick to the sphere when they encounter it. The average density near the sphere will decrease because the particles are constantly withdrawn by adsorption and soon a stationary density gradient will be set up. The density c as a function of r, the distance from the center of the sphere, is then

$$c(r) = c_\infty(1 - a^1/r)$$

where c_∞ is the average density at a great distance from the sphere, and a^1 is a constant which depends on the probability of adsorption once a particle comes close enough to the surface of the sphere for direct interaction. If every approach to the surface leads to adsorption the density near the surface must drop to zero and we must therefore have $a^1 = a$. If not every

approach is successful, the density near the surface will be positive and $a^1 < a$. This stationary density distribution is quite independent of the diffusion constant. It causes, however, a constant flow F towards the surface which is proportional to the density gradient and to the diffusion constant D."

The concentration of the substrate will thus increase centrifugally from the active center according to a hyperbolic curve. In other words, the "sphere of influence" will have no sharp boundaries. However, after a rather rapid initial change of concentration, we will come into a zone where further changes are extremely slow. If we set the limit of the sphere at an arbitrary concentration value of 0.9 (which is a reasonable assumption in view of the fact that there must be in the cell a margin of safety of substrate concentration), the calculation shows that this limit is reached at a distance of about ten times the radius of the active particle (which may be only a part of the gene, in case the gene, like most enzymes, has a prosthetic group) if every encounter of a substrate molecule with the gene is effective. If it is not, this value will be further reduced. Ten times the radius of the active particle is thus, on the above assumptions, our upper limit.

A rather similar result is obtained if the same reasoning is applied to the product P of gene activity (instead of S). For a spherical gene (or active group of the gene), acting at a uniform rate in a homogeneous medium containing no other sources of P, the equilibrium concentration

$$c = c_a \cdot \frac{a}{r}$$

where c_a is the concentration of P at the surface and the other terms the same as above, but in terms of P instead of S. The boundary of the "sphere of influence" (defined as comprising the concentrations of P between 1.0 and 0.1) is at a distance of the same order of magnitude as above, the gradient being in the opposite direction (c decreasing centrifugally).

Let us turn now to position effect. According to Demerec[13] position effect can extend over as far as fifty bands of the salivary gland chromosome. We will assume that a chromonema in the interphase nucleus of a somatic cell, although considerably shorter than the salivary gland chromosome, has the same proportions as the latter. We will visualize the genes as spherical bodies corresponding in position to the dark bands and the intergenic material to the light spaces. The gene then corresponds at the most to $^1/_{100}$ of the total length of the fifty band segment, and position effect can then cover 200 times its radius. By analogy with enzymes it is almost certain that the active part of the gene corresponds to only a small part of the gene molecule and, as it is further possible that one chromosome band contains more than one gene, it follows that the distance covered by

position effect in this case is at least 200 times the radius of the gene and probably more.

A different computation in actual Ångström units may be made without assuming a strict similarity between the salivary gland and interphase chromosomes. It is reasonable to assume for the active part of the gene a radius of an order not exceeding 5 Å. The length of the X chromosome at metaphase is about 2 μ. The euchromatic part is approximately 1.4 μ. As the X chromosome has about 1000 bands, 50 bands represents $^1/_{20}$ of this length, or 0.07 μ = 700 Å. The latter value is again an underestimate, because the interphase chromosomes are certainly less coiled, and numerous observations indicate that the genes are active during the interphase. It thus appears that unless we make some further hypothesis which would be unjustified in the present status of our knowledge (assuming, for instance, non-stationary states or interactions of a more complicated nature with substrates or products of other genes, or ascribing particular physiological importance, in the case of interaction of gene products, to the concentration changes outside the boundary of the "sphere of influence"), the "spheres of influence" do not extend far enough to offer an explanation for position effect.

While this reasoning is based on crude assumptions, and cannot therefore disprove the kinetic hypothesis, it is quoted here because it shows that the latter in its simplest form meets with the same difficulty in regard to distance as does the simplest form of the structural hypothesis. It should be pointed out in addition that *position effects are now known to extend over such great distances that it is difficult to see, on the basis of the kinetic hypothesis, why interactions similar to those which occur between two given genes in position effect should not occur between the same genes in their normal location, when the chromosome regions in which they are located happen by chance to lie close together in the nucleus.* Together with the difficulties which the kinetic interpretation encounters when its application is attempted to concrete cases (see below), these arguments reduce considerably its explanatory value. Moreover the italicized statement indicates that the explanation of the mechanism of position effect must be sought in terms of a *factor which will spread along or across the chromosomes rather than through the karyolymph. This characteristic suggests that the factor primarily responsible for position effect corresponds to a change in the physical state of the chromosome itself rather than to a change in the distribution of a substance emanating from it.*[16]

Before we center our attention on any one of the possible aspects of the physical condition of the chromosomes, it may be well to recall the close relationship between the enzymatic and mechanical properties of the muscle protein, myosin, revealed by the recent work of Engelhardt, *et al.*,[17, 18] Needham, *et al.*,[19, 20, 21] Bailey[22] and others. The state of extension of

myosin fibers appears to be directly linked to the substrate-enzyme combination, the enzyme (adenosinepyrophosphatase) activity being intimately associated with the fibrous protein itself and the substrate (adenosinetriphosphate) serving as source of the energy required in the process of relaxation. The contraction and extension of the myosin fiber are regarded as results of folding and unfolding of the polypeptid chain, the first of the two processes (folding) perhaps being caused directly by the enzyme-substrate association ("the molecular length of the adenylpyrophosphate being shorter than the length between the groups on the myosin chain to which it becomes attached"[20]).

The association in myosin of fibrous structure with enzyme activity cannot fail to evoke the analogy with the chromosome, with its obviously enzymatic (genic) function and cyclic mechanical changes. Several authors have visualized the chromosome as consisting of a polypeptide backbone, which remains the same throughout its length, and of different linked materials (groups of radicals) responsible for the longitudinal differentiation of the chromosome into somewhat different regions, the genes (compare Demerec[23, 24]).

It is by no means unlikely that in such a structure the state of extension will lead, through changes in spacing, to changes in function. That spatial relationship should play a decisive role in the enzyme substrate combination, the first step of enzyme action, is a safe assumption. That the state of extension (degree of denaturation?) may or may not, according to the case (nature of substance and method of stretching), have profound effects on the specificity and biological activity of a protein seems to be clearly indicated by the heterogeneity of the results obtained by different authors working with various proteins.[25] The important point in the present connection is that in some cases the state of extension *does* affect the biological function, and it may be said that "so long as a biological test for activity is used, a substance which is active after being spread may be active because the change brought about in spreading is reversed during the activity test" (Anson[26]).

Returning now to position effect, it will be noted that the intimate association of chromosomes in somatic pairing, the coincidence of which with the presence of position effect in Diptera has already drawn the attention of Muller[12] and Schultz,[27] may reasonably be assumed to lead to a more precise alignment of the submicroscopic structures than that which prevails in unpaired segments. That synaptic association may actually lead to a stretching out of the whole structure of the chromosome is conceivable and moreover suggested by certain cytological features of the salivary gland chromosomes. (It may be objected indeed that the inference from the pictures of salivary gland chromosomes to the interphase somatic chromosomes is hazardous. At present, however, this seems to be the only

possible procedure.) Finally, it is easy to see that the presence of chromosomal rearrangements may lead, through changes in the pairing condition, to modifications of the state of stress in the immediate vicinity of breakage points.

We would like to emphasize that on this scheme in which the change in gene function in position effect is regarded as a result of changed conditions in the chromosome segment concerned, the normal association of the unaffected parts of the chromosome is emphasized as much as, or more than the eventual absence of it in the affected part. Although this may appear as only a minor change of emphasis, it appears important to us (1) because it centers the attention on the established fact of pairing (whatever its mechanism) instead of on the "forces which, in the case of homologous genes, bring about their synaptic association" and which when "acting between unlike genes, should tend to deform them" (Muller[12]); (2) because, if viewed from this angle, the suggested mechanism is not confined in its action to the genes in the chromosome carrying the break. It applies as well to its homologue. The importance of this point will appear when we come to the discussion of a few cases of position effect in the light of the above scheme.

It is clear to us that, in its present form, the scheme offered for the explanation of position effect is too simple to account for all cases which are classified today as position effects. We are aware that it will require at least further refinements, if not remodeling, and we shall refer later in this paper to the difficulties that it encounters at once. First, however, we want to try its application to a few cases which appear particularly relevant.

As pointed out by Stern, to whom we owe the only detailed account of the dosage relationship at a locus which is profoundly affected also by changes in position, these two properties have to be considered together if one wants to test any explanation for the latter. The data of Stern[28] and Stern and Schaeffer[29,30] on the dosage relationships of different wild-type and mutant alleles at the *ci* (*cubitus interruptus*) locus in the normal chromosome 4 have been interpreted by them in terms of overlapping "spheres of influence" of the homologous genes across the chromosomes. The effects obtained could be explained by assuming that different alleles possessed different capacities (1) for combining with a common (limited) substrate, and (2) for converting it into a substance actively contributing to the normal phenotype; so that, for instance, a gene with a high capacity for combining with the substrate and a low efficiency in converting it would interfere with the activity of a more efficient one by depriving it of substrate. Such a hypothesis, as we have seen, can be extended to position effects at the *ci* locus where interference may be assumed between non-homologous genes along the same chromosome, as well as between homologues across the chromosomes. Decrease of dominance of ci^+ in a translocated chromosome

[in the combination $R(ci^+)/ci$][31] would thus be due to interference along the chromosome, reducing the efficiency of the ci^+ allele. But in experiments on position effects of the recessive *ci* allele, Stern and Heidenthal[31] have found, first, that the effect of a + allele in the normal chromosome over a translocated *ci* allele ($R(ci)/+$) deviates more from normality than that of a + allele alone (in haplo-4); and second, that the combination $R(ci)/R(ci^+)$ has a more normal phenotype than the combination $R(ci)/+$. While the first of these facts by itself might be explained on the basis of competition across the chromosomes, this interpretation is inconsistent with the second finding, namely, that although the phenotypes of both $R(ci^+)/ci$ and $R(ci)/+$ are less normal than that of $+/ci$, the combination of the two translocations [$R(ci^+)/R(ci)$] partly restores the normal activity instead of decreasing it still more.

Stern and Heidenthal[31] thus reach the conclusion that "many, but not all, facts reported can be interpreted in terms of concepts regarding genic action which have recently been proposed." The same conclusion regarding kinetic interpretations has been derived above from quite a different line of reasoning. This situation obviously requires the introduction of a new parameter, independent of the properties of the genes which were introduced to account for the dosage relations.

The mechanical hypothesis presented here requires no special parameter for the last discussed case as will be seen by its application in the following paragraphs. In Stern's case of heterozygotes for a fourth chromosome rearrangement and a normal fourth [among which $R(ci^+)/ci$ and $R(ci)/+$ are included] the normal pairing of the fourth chromosomes is modified, and the relationships of the chromosomes are such that there may be modification of stress on either the translocated or the normal chromosome, or both, in the region of the break.

As regards $R(ci^+)/R(ci)$ the actual pairing conditions between the two translocations are not known, and this makes it impossible either to predict the phenotype on the basis of the same assumption of modification of stress or to say whether the observed phenotype agrees with this assumption. Indeed, these conditions may be very different from those obtaining in heterozygotes for one translocation, and might be more analogous to those of a translocation homozygote or hemizygote.

The situation shown by data of Dubinin and Sidorov[32, 33] on translocations of ci^+ is much clearer. Any of these translocations which gave rise to position effects when heterozygous ($R(ci^+)/ci$) were found to give a completely normal phenotype when homozygous or hemizygous. *These results suggest that structural heterozygosity might be a necessary condition for the position effect to become effective, and that in the complete absence or complete normality of pairing in the region of the affected locus the position effect is only potential.*

Dubinin and Sidorov also found that flies heterozygous for two different translocations (each separately producing a position effect when heterozygous with a normal *ci* chromosome) were wild type in appearance. It is possible that the chromosome configurations in these heterozygotes correspond in general features to that in the $R(ci^+)/R(ci)$ combination.

The change of dominance of Punch reported by Oliver[34] is another case in point. Punch is associated with a translocation and is dominant when heterozygous with normal. This effect can be explained as a reduction of the normal activity of the Pu^+ alleles in both chromosomes, owing to the change in homology along the chromosome, and the resulting change of tension imposed by synaptic attraction towards a different partner on each side of the locus. *Pu* becomes recessive, however, when heterozygous with another translocation (T(2:4)A 34) in which one break is close to the *Pu* locus. The effect of the second translocation is to increase the normal activity of the *Pu* allele in the opposite chromosome, and we should therefore expect to find that the combination of the two translocations facilitates more normal pairing in the *Pu* region.

As Stern and Heidenthal[31] have indicated, an anomaly of the brown-variegated position effects can be eliminated by postulating a factor capable of affecting both chromosomes of a pair. The brown-variegated types are exceptional in that the effect is dominant, and it was previously necessary to assume that the translocated bw^+ locus is solely responsible for the dominant effect, having acquired a rather exceptional *antimorphic* activity (Muller[7]). On the structural interpretation, however, the bw^+ allele in the normal chromosome of a heterozygote may also be affected, so that the combined activity of the two alleles may be reduced in some cells below the level required for a wild type phenotype. We would infer, moreover, from the premise that the activity of two bw^+ alleles is so markedly reduced, that dominant position effects would be obtained even more readily from rearrangements with breaks in the neighborhood of a recessive *bw* allele; for in the $T(bw)/+$ heterozygote we should expect the activity of the + allele to be affected as much as in $T(bw^+)/+$, while the *bw* allele in the former heterozygote already has a reduced activity as compared with the translocated bw^+ in the latter. Although *bw* stocks have probably been irradiated rather infrequently, one such position effect is known (bw^{v6}, Glass[35]). The heterozygote $bw^{v6}/+$ shows variegation as a dominant effect as would be expected. Unfortunately no homozygotes for bw^{v6} were obtained before the stock was lost by reversion. Finally, it is of some interest that Schultz[36] found the Plum (dominant brown-variegated) effect to be suppressed in hybrids of *D. simulans* × *D. melanogaster*. Since it had previously been found by Kerkis[37] that synapsis of the salivary gland chromosomes is considerably reduced in these hybrids, the question may be

raised whether there is not some causal connection between the two phenomena.

In considering the case of brown variegation it is of interest to notice that, as compared with other position effects, variegated types are characterized by the fact that the change in function of a gene near to a break is expressed to a variable degree in different cells or groups of cells within the same organ. It appears possible to us that this variability will find its explanation on the basis of the same general interpretation here advanced, i.e., as due to variations in stress depending on the particular configurations of the chromosome complement in individual cells. We here find ourselves in close agreement with Muller,[1] when he says: "the degree and the character of gene functioning should also be influenced to some extent by the manner of grouping of the different chromosomes and chromosome regions with regard to each other, i.e., by the (partly accidental) mode of juxtaposition of different portions of the chromonema in the cell. Hence, in different somatic cells of the same individual, there might be some differences in gene functioning, depending upon the way the chromosomes happened to be situated. It is possible that some cases of somatic variegation may be caused in this way. In that case agents, genetic or otherwise, which affected chromosome arrangements, would thereby affect gene expression. A possible case in point might be the influence of the Y chromosome of Drosophila upon somatic variegation."

As the position effects discussed so far are all associated with breaks near the chromocenter, it is of interest to consider one of a somewhat different type. The Bar eye is due to a duplication in tandem of a euchromatic region. At first sight Bar (and the comparable position effects *Confluens Star, Hairy-wing*) may appear not to fit the hypothesis, because the phenotype is changed not only in heterozygotes, but also in homo- and hemizygotes. It is known, however, that in the Bar salivary gland chromosome there seems to be a tendency towards synapsis between homologous bands in the two duplicated sections so that even in homo- or hemizygous Bar both the outline of the chromosome and the details of banding are visibly distorted. It is clearly possible that a modification of stress may be occasioned in one part of the chromosome by synapsis between homologous genes along the chromosome as well as by the interchromosomal forces. Thus the difficulty presented by Bar may be only apparent.

A real difficulty must now be considered, however, in connection with the position effects of the w^+ and bw^+ loci. Rearrangements giving position effects of the w^+ locus when heterozygous, still produce the white-mottled phenotype in the homozygous or hemizygous condition (when these are viable), where there is either complete pairing or no pairing at all. The same is true of the brown-variegated homozygotes. Indeed, the latter deviate more from normality than their corresponding heterozygotes [38]

These facts appear to be reconcilable with the above scheme on the assumption that the manifestation of the position effect even in homozygous flies depends on the attachment of the affected loci to heterochromatic regions.

That the pairing conditions of heterochromatic regions differ from those of euchromatin is obvious from their aggregation in the chromocenter. In order to make clearer the possible relation between this so-called "non-homologous" pairing and position effect, a brief discussion of some other properties of heterochromatin is necessary.

Several lines of evidence lead one to suspect that the physical state of the chromocentral regions differs from that of euchromatin. This evidence may be summarized as follows: (1) The frequency of crossing-over per unit cytological length is lower in the neighborhood of the centromeres than elsewhere. This is apparently a function of proximity to the centromere, and not (or not altogether) an intrinsic property of heterochromatin (Mather[39]). It is interesting to note, however, that high temperature increases crossing-over in heterochromatin, irrespective of its position with regard to the centromere, to a level more nearly corresponding to that found in the euchromatin.[39] High temperature also modifies white-mottled and other position effects associated with heterochromatin towards wild-type, and this suggests that the same change in physical state that results in increased crossing-over is also responsible for the increased activity of the loci manifesting position effects. (2) The increase in length of heterochromatic regions in the salivary gland chromosomes as compared with mitotic prophase chromosomes is relatively less than the corresponding increase of euchromatic regions. This might be interpreted as meaning that the proteins of heterochromatic regions are already relatively extended, whereas those of euchromatin are much folded and therefore capable of greater stretching. (3) According to Caspersson[40,41] heterochromatin and euchromatin give different absorption curves in the ultra-violet region of the spectrum. This difference was held to mean that the protein constituents of heterochromatin were histones, while those of euchromatin were partly of the more complex, and more highly folded, globulin type. The validity of this particular interpretation is open to question (Mirsky and Pollister[42]), but if the differences in absorption are real there must be some underlying structural or chemical difference.

Returning now to the "non-homologous" pairing which heterochromatic regions exhibit, it may be pointed out that if the forces responsible for this pairing are operative between any two heterochromatic regions, they must act not only between regions in non-homologous chromosomes, but also between heterochromatic bands in the same chromosome. In this case, pairing conditions must be very similar to those associated with the Bar position effect. It is true that the Bar locus lies within a region of abnormal pairing, while genes concerned in heterochromatic position

effects lie outside of such a region, and cannot be directly affected by the conflicting synaptic forces. But if one assumes a difference (for instance, relative inextensibility) in heterochromatic structure on the grounds discussed above, one might expect changes in stress due to non-homologous pairing to be distributed along the chromosome and to affect the folded regions more than the relatively unfolded.

It must be emphasized that this explanation is only tentative, and until experimental proof of such a situation is available, this will remain the most serious difficulty confronting the general interpretation. Even if this difficulty is set aside, we are aware that the interpretation proposed is entirely hypothetical. The justification for presenting it is that it is open to verification in a number of ways, some of which may now be considered.

It has already been mentioned, in referring to some examples of position effect, that without a precise knowledge of their cytology, the results can only be interpreted provisionally. One of the tests to which the hypothesis here presented is subject is a detailed study of the synaptic configurations characteristic of these chromosomal rearrangements. According to the hypothesis, if we compare aberrations which give rise to position effects with those where no phenotypic change is apparent (for example, the two combinations of Punch described by Oliver[34]), we should expect to find that these differ in some respect such as the degree of synapsis or the variability of the synaptic configuration from cell to cell.

It should also be possible to demonstrate position effects across the chromosomes in a number of cases where, as in *R(ci)*, aberrations are produced with breaks in the neighborhood of recessive alleles. Especially if such an allele were an amorph, a reduction in the activity of the gene in the opposite (normal) chromosome might be expected to result in a deviation from the normal phenotype. The *bw* locus was mentioned as a case in point. Preliminary experiments involving the *w* locus have yielded results which are not incompatible with the interpretation proposed.

A third type of support for the hypothesis might be derived from studying correlatively the effects of different factors on pairing and on the degree of expression of position effects. For instance, non-disjunction may be considered a result of abnormal meiotic pairing; and marked modifications of position effects following non-disjunction have been observed by the junior author. A common causal agent may be responsible for both phenomena, although this has not yet been demonstrated and another interpretation is possible. The above-mentioned absence of pairing in Drosophila hybrids also seems to offer a field for experimentation.[43]

Summary.—The hypotheses on the mechanism of position effect are critically reviewed. An interpretation is offered which regards position effect as a result of chromosome pairing that causes a modification of stress near the affected loci. Several known cases of position effects are dis-

cussed in the light of this interpretation and methods of testing it are suggested.

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[36] In Morgan, T. H., Bridges, C. B., and Schultz, J., *Yearbook Carnegie Inst.*, No. 36, 298–305 (1937).

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[38] It is possible, however, that this deviation is somewhat misleading, for the change in phenotype may be broken down into two components—a change from wild type to brown and a change from homogeneous eye color to variegated. The former change is more marked in homozygotes than in heterozygotes, but the latter is diminished.

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[43] This paper was read prior to publication by Drs. M. Delbrück, Th. Dobzhansky, H. B. Glass, B. McClintock, H. J. Muller, C. Stern and several members of this department to whom we wish to express our thanks.

MATERIALS FOR THE STUDY OF THE POSITION EFFECT OF NORMAL AND MUTANT GENES

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The majority of recent studies on the position effect of genes in *Drosophila* have been concerned with normal alleles of various loci. Different kinds of effects seem to have resulted from change in position. In many cases the phenotypic expression of a normal gene has not been altered by a change of position. Some cases, however, have been observed in which the normal allele assumed a dominant mutant action (e.g., the dominant eye color, Plum). Other observations have indicated that the dominance of a normal allele may be diminished (e.g., cubitus interruptus), or that a normal allele may become more or less inactivated (e.g., white and Notch series). Few generalizations could be made from these results. It was thought, therefore, that some insight might be gained from a study which would attempt to correlate data derived from investigations of change in position of both a normal gene and its mutant alleles. The following represents a first report on such studies.[1]

Material.—The locus chosen was that of cubitus interruptus (*ci*) in the fourth chromosome of *D. melanogaster*. In 1934 Dubinin and Siderov[2] showed that many translocations involving the fourth chromosome cause an unusual change in the effect of the normal allele originally present. While this allele in its new position, both in hemi- or homozygous constitution, still produces a normal phenotype, it causes in heterozygous combina-

tion with the recessive mutant allele, *ci*, the appearance of the mutant vein character *ci*, an interruption of the cubital vein.

In order to obtain an unselected sample of translocations involving the fourth chromosome and the mutant allele *ci*, males homozygous for *ci* were x-rayed at 4000 r,[3] and crossed to females containing dominant marker genes in their second and third chromosomes. More than 20,000 F_1 males were individually back-crossed to *ci* $ey^R/ci\ ey^R$ females in order to test for the presence of translocations.[4] Fifty-five translocations involving the fourth chromosome and one or more of the chromosomes *Y*, II or III were found, and stocks of all of them established. Heterozygotes were obtained between all translocations and the allele *ci* in its normal position. About half of the translocations proved to give a more extreme phenotype in combination with *ci* than the typical mutant homozygote *ci*/*ci*. Five translocations of this group form the basis of the experiments to be reported. These five translocations were selected among those which, during an early stage of these studies, were found to exhibit the position effect described in the following section.

We shall use the symbol *R*(*ci*) for any rearrangement (*R*) which leads to a position effect of the mutant allele *ci*. Correspondingly *R*(+) will be used to denote a rearrangement giving a position effect of a normal allele +. *R*(*ci*) and *R*(+) may be referred to as "position alleles" since they behave as alleles of genes like *ci* and + in the typical position.

Heterozygotes of Position Alleles R(ci) and a Normal Allele.[5]—It was discovered that at room temperature some of the heterozygous $R(ci)/+^C$ individuals showed slight degrees of the *ci* phenotype, in contrast to $ci/+^C$ heterozygotes which at this temperature range are all normal.[6] In order to obtain reliable data, heterozygotes were raised at a lower temperature, 18–19°, which is known to intensify the expression of the *ci* phenotype. Table 1 shows that all five position alleles *R*(*ci*), in combination with $+^C$, gave a larger number of individuals with the mutant phenotype than did $ci/+^C$ flies. Furthermore, while no individual of the latter consitution deviated more from normality than Class 0, which still shows uninterrupted though thinned veins, many flies of the constitution $R(ci)/+^C$ belonged to the more extreme Classes 1, 2 and 3.

The five translocations differ in the intensity with which they cause the mutant phenotype. For the present, two main groups of *R*(*ci*) alleles will be distinguished: (1) *R*(*ci*) –8 and –48 in which the phenotypic distribution of the heterozygotes $R(ci)/+^C$ deviates rather slightly from that of $ci/+^C$, Class *N* still representing 80 per cent or more of all individuals; and (2) *R*(*ci*) –23, –29 and –32 in which the deviations from normality are larger with only 60 per cent or less of the individuals belonging to class *N*.

Data from a preliminary cytological analysis of these translocations,

undertaken by Dr. H. D. Stalker, are included in table 1. Of the two translocations with weak expression one occurred near the chromocenter of chromosome 3 and one near the middle of 3*L*, while all three translocations with strong expression were located within close range of each other, about one-third the distance from the distal end of 3*R*. All breaks in chromosome 4 presumably lie proximal to 101*F*, i.e., between the centromere and the locus of *ci*, as described for the *R*(+) position alleles by Khvostova and Gavrilova (1935).[7]

Heterozygotes of Position Alleles R(ci) and ci.—As stated above, the five *R*(*ci*) translocations were chosen from those which in the combination *R*(*ci*)/*ci* produce a more extreme *ci* phenotype than the non-translocated *ci*/*ci* homozygote. Specific data permitting a comparison between *R*(*ci*)/*ci* and *ci*/*ci* sibs will be reported elsewhere, but in summary it may

TABLE 1

F_1 NON-ey^D OF WILD TYPE "CANTON" $(+^C/+^C) \times ci\ ey^R/ci\ ey^R$ OR $R(ci)/ey^D$* 18°C.

HETEROZYGOUS FOR $+^C$ AND:	BREAK IN CHROMOSOME 3 NEAR SALIVARY CHROMOSOME REGION:	PHENOTYPIC CLASSES† ♀					♂				
		N	0	1	2	3	*N*	0	1	2	3
ci ey	...	484	1	..	.	..	458	18	..	.	..
R(*ci*) – 8‡	69/70	129	16	5	.	..	157	16	5	.	..
R(*ci*) – 48	Chromocenter	174	10	..	.	..	153	17	..	.	..
R(*ci*) – 23	92 *E*	2	13	21	.	..	40	20	5	.	..
R(*ci*) – 29	94 *A*	21	11	28	4	..	11	26	21	2	..
R(*ci*) – 32	92 *E*	38	33	55	9	..	15	17	58	6	..

* ey^D = eyeless-dominant; chromosome IV.

† *N* = uninterrupted distal section of 4th vein; 0 = uninterrupted but thinned section; 1, 2 = different progressive degrees of interruption; 3 = absence of whole section.

‡ The *R*(*ci*) alleles are numbered in chronological order of their discovery.

be stated that the maximum of the frequency distributions of venation classes in *R*(*ci*)/*ci* are shifted to the next, more extreme class or even farther. Table 2 (right column) shows that the five position alleles differ in the degree to which they cause the expression of the *ci* phenotype in the combination *R*(*ci*)/*ci*. It is possible to distinguish two groups of *R*(*ci*) alleles, one leading to a distribution of phenotypes around a mean located between Classes 1 and 2, and another with the more extreme mean between Classes 2 and 3. Comparing the degree of effect of the *R*(*ci*) alleles in *R*(*ci*)/+ (table 1) with *R*(*ci*)/*ci* (table 3), it is seen that a strong effect in the former constitution is paralleled by a strong effect in the latter and vice versa.

Heterozygotes between Two Position Alleles R(+) and R(ci).—A translocation N^{264-20} between the first and fourth chromosome, produced by x-radiation of a fly with normal alleles of *ci*, was obtained through the

courtesy of Dr. M. Demerec.[8] Females heterozygous for this translocation show a Notch (N) phenotype. They were crossed to *ci* males. Among the offspring those which were heterozygous for the translocation and *ci* exhibited a typical "Dubinin effect": Their phenotype showed *ci* venation with a maximum at Class 2 (table 3). Thus, the N-translocation may be labeled as possessing a position allele $R(+)$.

Females of the constitution $R(+)/ci$ were mated to males which carried one of the five tested $R(ci)$ alleles and, as a marker for their non-translocated fourth chromosome, the dominant ey^D. The non-ey^D female offspring of these crosses were either $R(ci)/ci$ (table 2, right column) or $R(+)/R(ci)$ (table 2, left column). In all five cases the $R(+)/R(ci)$ flies were more normal than their $R(ci)/ci$ sibs (right column) or than $R(+)/ci$ flies (table 3). Indeed, three different types of $R(+)/R(ci)$ flies were nearly

TABLE 2

F_1 NON-ey^D ♀♀ OF *y* $N^{264\text{-}20}$ B^iB^i; $R(+)/ci\ ey^R \times R(ci)/ey^D$ 26°C.

	$R(+)/R(ci)$					$R(ci)/ci\ ey^R$				
	N	0	1	2	3	*N*	0	1	2	3
$R(ci)$ – 8	37	12	24	17	1	3	7	27	18	1
$R(ci)$ – 48	35	12	31	29	4	.	8	45	62	6
$R(ci)$ – 23	15	1	..	..	.	.	1	1	12	5
$R(ci)$ – 29	48	1	..	4	.	.	.	4	16	20
$R(ci)$ – 32	50	..	..	..	.	.	.	3	16	25

TABLE 3

F_1 ♀♀ OF *y* $N^{264\text{-}20}$ B^iB^i; $R(+)/y\ Hw\ m^2\ g^4\ dl\text{-}49$ * $\times$ *ci* $ey^R/ci\ ey^R$ 26°C.

$R(+)/ci\ ey^R$					$Hw/ci\ ey^R$	
N	0	1	2	3	*N*	0–3
..	4	40	186	27	275	..

* *y*, *Hw*, B^iB^i, m^2 and g^4 are sex-linked factors which segregate together as a unit due to the presence of *dl*-49, an inversion, and of $N^{264\text{-}20}$.

completely normal. Moreover, the curious relation is apparent that those $R(ci)$ alleles which cause an extreme *ci* phenotype in either combination $R(ci)/+$ or $R(ci)/ci$ produce the least extreme expression in $R(ci)/R(+)$.

It will be necessary to test different $R(+)$ alleles with the $R(ci)$ alleles recorded in table 2, and with others, in order to find out how general are these unexpected results. We have crossed three of the $R(ci)$ alleles used in combination with the $R(+),N^{264\text{-}20}$, with another $R(+),w^{258\text{-}18}$. This latter translocation does not show abnormal "Notch" wing characters as does $N^{264\text{-}20}$. Nevertheless, the $R(+),w^{258\text{-}18}$, gave equivalent results in combinations with $R(ci)$, indicating that the approach to normal, non-*ci* venation in $R(+)/R(ci)$ is independent of the specific phenotype caused by Notch.[9]

Discussion.—When Dubinin and Siderov[2] found that $R(+)$/haplo-IV

and $R(+)/R(+)$ produce normal phenotypes while $R(+)/ci$ flies have interrupted veins, it seemed that such relations between alleles were unique for the position effect. It has since been shown (Stern 1943)[10] that the action of *ci* alleles may be described in terms of combining ability, *c*, with a cellular substrate, *S*, and efficiency, *e*, in transforming *S* into a product, *P*, effective in leading toward a normal phenotype. With the help of these concepts the Dubinin effect can be understood if it is assumed that there is a sufficient amount of substrate and that the $R(+)$ allele has sufficient combining power and efficiency in homo- or hemizygous constitution to cause normality; that in the heterozygote $R(+)/ci$ there is competition for *S* between the $R(+)$ and *ci* alleles, and that the efficiency of *ci* is less than that of $R(+)$, and hence the amount of *P* formed is below normal. Similarly the phenotypic effect of $R(ci)$ in the combination $R(ci)/+$ can be understood as the outcome of competition between $R(ci)$

TABLE 4

POSSIBLE CHANGES OF GENIC ATTRIBUTES

	COMBINING POWER *c*	EFFICIENCY *e*
1	Unchanged	Lower
2	Unchanged	Higher
3	Lower	Unchanged
4	Higher	Unchanged
5	Lower	Lower
6	Lower	Higher
7	Higher	Lower
8	Higher	Higher

and + in which the former deprives + of *S* and transforms *S* into *P* at a low efficiency. If the effects of the changed position of the + and *ci* allele are described in terms of dominance and recessiveness, the change from + to $R(+)$ as seen in $R(+)/ci$ versus $+/ci$ would be called a *decrease* of dominance of the + allele while the change from *ci* to $R(ci)$, as seen in $R(ci)/+$ versus $ci/+$ would be an *increase* of dominance of the *ci* allele. The interpretation of position effects in terms of competition resolves the apparent paradox.

In the preceding paragraph the interaction of the various alleles has been considered separately for certain allelic combinations. This led to the recognition of competition between alleles, and of the relative efficiencies of + as compared with *ci*, of $R(+)$ as compared with *ci*, and of + as compared with $R(ci)$. It is now appropriate to ask in which way the position allele $R(+)$ differs from the original allele +, and $R(ci)$ from *ci*. If the amount of available substrate *S* is the same for the gene in its altered as in its original position, then all changes in effect due to new positions of alleles will be the result of changes in the combining power,

c, or of the efficiency, *e*, or of both. We shall begin our analysis with this assumption of constancy of *S*. Variability of *S* due to changed position of alleles will be considered later. Since it is possible *a priori* that either *c* or *e* may remain unchanged, or be increased or decreased there are eight combinations possible in which at least one of the two properties is altered (table 4). Considering that there exists competition in the heterozygote $+/ci$ between $+$ and *ci* the decreased effect of $R(+)/ci$ could be brought about by any one of the following changes of the $R(+)$ allele as compared with $+$: (1), (3), (5), (6) and (7). On the other hand, the decreased effect of $R(ci)/+$ could be produced by any one of the following changes of the $R(ci)$ allele as compared with *ci:* (1), (4), (5) and (7). Since it is reasonable to assume that the type of change caused by the altered position is the same, in general, for $R(+)$ and $R(ci)$ three possibilities which account for changes in only one of either $R(+)$ or $R(ci)$, but not in both, can be eliminated, namely (3), (4) and (6). This leaves (1), (5) and (7). These three types of changes all agree in that they involve a lower efficiency of the position allele as compared with the original allele. They differ in the type of *c* attribute which may be unchanged, lower or higher than in the original allele. No information is available at present which permits a decision as to which of these three possibilities is actually realized. Any one of them is not only compatible with the effect of $R(+)/ci$ and $R(ci)/+$ but also with the lowered effect of $R(ci)/ci$ as compared with ci/ci. Competition, with low efficiency of a position allele, accounts not only for the "dominant" mutant effect of $R(ci)$ but may provide a general explanation for the occurrence of dominant effects in loci which otherwise have produced recessive mutants only (e.g., Plum).

It will elucidate the problem further to discuss briefly in what ways a new position may cause a change in the effect of a gene.[11] If genic action consists of a primary interaction with a substrate, *S*, leading to a product, *P*, one may conceive of this interaction as being composed of two separate steps. The first would consist of a reaction leading to a gene-substrate association, the second to restoration of the gene with release of *P*. The term combining power would apply specifically to the first reaction and be equivalent to an association constant. The term efficiency might be equivalent to the velocity constant which characterizes the second reaction, provided the gene products *P* of the different alleles are alike or at least equivalent in their effect. If the gene products are different and have different values in leading toward a given phenotype, then the efficiency, *e*, of an allele is a function of both the velocity constant and of the effectiveness of its specific *P*. The deduction—made under the unproved assumption of constancy of *S*—that the observed position effects at the *ci* locus involve a lowered efficiency, localizes these effects on the second step of the gene-substrate interaction. Whether changes in the first step of this re-

action are also involved remains undecided. This analysis of the position effect, however, leaves open the question as to the primary change responsible for the changed reaction. The primary change may be an alteration of actual genic structure, or some kind of alteration due to unusual synaptic forces (Muller, 1935).[12] It may even not involve any alteration of the gene itself but may be due either to a changed local environment under which the reactions must proceed or due to a changed amount of appropriately localized and accessible substrate. If in the new position less substrate were available than originally, a lowered total effect would result in the heterozygote of a position allele and the allele in the normal chromosome. By making, in this case, the unjustified assumption of an unchanged amount of S the lowered effect would be mistakenly interpreted as due to changes in the c and e attributes. An example may clarify this point. Be it assumed that the $R(+)$ and $R(ci)$ positions involve a reduction of available S. Since, in $R(+)/ci$, $R(+)$ and ci share in S the actual amount of S available not only to $R(+)$ but also to ci might be diminished. Similarly, in $R(ci)/+$, the amount of S available not only to $R(ci)$ but also to $+$ might be diminished. If, without knowledge of the decrease in S, the amount of the latter, as well as the c and e properties of the normally located alleles ci and $+$ were regarded as unchanged, then the change in total P produced by $R(+)/ci$ and $R(ci)/+$ would appear as caused by a lowered e of $R(+)$ and $R(ci)$ (with or without change of their c). In the absence of information as to the amount of available S in heterozygotes for position alleles as compared with typical heterozygotes, we may summarize our analysis by stating that competition between the alleles is involved in the case of position effects of the ci locus as it is in typical heterozygotes but that the primary cause or causes of the difference in effect of position heterozygotes as compared with typical heterozygotes remains an open problem. Such a primary factor may either be a diminished amount of available substrate, a decreased efficiency of the position alleles, or possibly a combination of both.

While the proposed interpretation in terms of competition of the position alleles seems to unify most facts concerning gene action of typical and position alleles, it is unsuccessful without additional hypotheses in accounting for the effect of the combination $R(+)/R(ci)$. Since both $R(+)/ci$ and $R(ci)/+$ cause an effect less normal than $+/ci$, it was expected that $R(+)/R(ci)$ would be still less normal. Instead, this constitution causes a shift, in some cases nearly complete, toward normality. Possibly this fact is related to the type of somatic pairing occurring in heterozygotes for two translocations as compared to heterozygotes for only one translocation. No satisfactory interpretation, however, can be proposed at present.[13] It may be added that an apparently similar case has recently been described in an abstract by Oliver (1943),[14] though his inter-

pretation differs from the one given for *ci*. He found that the "dominant" eye color Punch which arose in connection with a translocation becomes "recessive" when combined with another translocation in the homologous chromosome which carries a normal allele.

Incidentally, the data reported furnish a unique example of the reality of "premutation," or "directed mutation" as postulated frequently in the early genetic discussions of evolutionary phenomena. The three "alleles" +, *ci* and *R(ci)* according to their effect may be described as dominant normal, recessive mutant and dominant mutant. A dominant mutant allele at the *ci* locus has only twice been found to be due to a single-step mutation from a normal allele. Our data show that such dominant mutant "alleles" can be easily produced by a step involving change of the effect of the recessive mutant *ci* which itself is removed by one mutational step from +.

Summary.—In *D. melanogaster* five translocations between the third and fourth chromosome, carrying the mutant allele *ci*, were studied. In combination with an unbroken fourth chromosome containing a normal allele they produce, in varying degrees, *ci* phenotypes in contrast to the typical heterozygote +/*ci* which appears nearly normal. Combined with *ci* in an unbroken fourth chromosome they lead, in varying degrees, to more extreme *ci* phenotypes than *ci*/*ci*. The three translocations which cause the greatest deviation from normality in combination with + also do so in combination with *ci*. A translocation between chromosomes 1 and 4 derived from a stock containing a normal allele of *ci* produces a striking *ci* phenotype if combined with *ci* in an unbroken chromosome ("Dubinin effect"). If this translocation instead is combined with *ci*, as carried in any one of the five translocations, a shift of phenotypes toward normality occurs. Those translocations which cause the least normal phenotypes in combination with + and *ci* are most effective in causing a shift toward normality when combined with the +-carrying translocation.

Many, but not all, facts reported can be interpreted in terms of concepts regarding genic action which have recently been proposed.[10] Other facts seem to involve additional phenomena. The position effects in heterozygotes appear as competition between the alleles in the normal and changed position.

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[1] An abstract had been submitted to the Genetics Society for the 1942 meeting; see Stern, C., *Genetics*, **28**, 92–93 (1943).

[2] Dubinin, N. P., and Siderov, B. N., *Am. Nat.*, **68**, 377–381 (1934).

[3] We wish to acknowledge the kindness of Dr. S. W. Warren, Professor of Radiology, The University of Rochester School of Medicine and Dentistry, who carried out the radiations.

[4] ey^R, a recessive allele of eyeless, served as a marker gene for non-radiated fourth chromosomes. It is without influence on the expression of *ci*. Except in the tables, reference to ey^R will be omitted.

[5] The normal allele was the $+^C$ allele of the *ci* locus, see Stern, C., and Schaeffer, E. W., *Proc. Nat. Acad. Sci.*, **29**, 361–367 (1943).

[6] After completion of the manuscript of the present communication a paper by Siderov, B. H., *Compt. rend. acad. sci. U.R.S.S.*, **31**, 390–392 (1941), has come to attention in which similar effects of $R(ci)$ alleles in combination with + are described.

[7] Khvostova, W. W., and Gavrilova, A. A., *Biol. Zhurnal.*, **4**, 905–916 (1935).

[8] "Salivary chromosomes (Sutton) show break in X at 3 C, with deficiency of 3C 5–7; and break in 4 at 101F," Bridges and Brehme, "The Mutants of *Drosophila melanogaster*," *Carnegie Inst. Wash. Publ.*, **552** (1944).

[9] In the paper of Siderov referred to in footnote 8 a further remarkable fact is reported. Two translocations between the third chromosome and a fourth chromosome which carries a + allele but which do not give a Dubinin effect in combination with *ci*, shift the phenotype toward normality if combined with $R(ci)$.

[10] Stern, C., *Genetics*, **28**, 441–475 (1943).

[11] Grateful acknowledgment is made to Dr. D. R. Goddard for clarification of some of the concepts involved in this discussion.

[12] Muller, H. J., *Summ. Commun. XV int. physiol. Congr.* (Leningr.-Mosc.), pp. 286–289 (1935); and *Proc. 15th int. physiol. Congr.* (Leningr.-Mosc.), pp. 587–589 (1938).

[13] If it were true that the new positions imply a reduction of S at the *ci* locus and if heterozygotes between normally located and position alleles frequently undergo somatic pairing while compounds between $R(+)$ and $R(ci)$ rarely pair, then competition might take place between the paired alleles of heterozygotes, while no competition might occur between the separated alleles $R(+)$ and $R(ci)$. The lack of competition might then permit a shift toward normality. The assumptions made are liable to experimental test.

[14] Oliver, C. P., *Anat. Rec.*, **87**, 33 (1943).

COMPARISON OF A DEGENERATE FORM OF EINSTEIN'S WITH BIRKHOFF'S THEORY OF GRAVITATION

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Whereas electric charge is not universally proportional to the inertial mass of bodies their gravity is. This fundamental fact, supported both by daily experience and the most refined experiments, led Einstein to the conception that inertia and gravitation are one (principle of equivalence) and thus to his theory of general relativity. The main reason for my and many others' belief in that theory is the radical explanation it affords for the fact just mentioned. Any theory which breaks up the unity of inertia and gravitation, as Birkhoff's recent theory of gravitation in a flat world[1] does, throws us back into the position before Einstein where we had to

accept the identity of mass and weight without understanding it. Is there any reason for such a withdrawal?

One way of breaking the bond between inertia and gravitation is suggested by the study of weak gravitational fields in Einstein's theory: One lets the g_{ik} differ but infinitesimally from constant values δ_{ik}, $g_{ik} = \delta_{ik} + \epsilon h_{ik}$ (δ_{ik} = inertia, h_{ik} = gravitation), throws away all higher powers of the constant factor ϵ except the first, writes h_{ik} for ϵh_{ik} and adopts the equations thus obtained as strictly valid. In a review[2] of Birkhoff's paper I had tried to illustrate his scheme by analogy with this "degenerate Einstein theory." In a recent note[3] A. Barajas claims to disprove the validity of this comparison. But he misinterprets my intention, and his calculations are not entirely correct. I hope to clarify the situation by the following fuller statement of the case, in which I compare the complete and the "degenerate" Einstein theories (E, D) with the theory of Birkhoff (B).

In terms of a suitable parameter σ the equations of the world trajectory of a particle have the common form

$$\frac{d^2x_i}{d\sigma^2} = -\gamma \cdot \Gamma^i_{pq} u^p u^q \qquad (u^i = dx_i/d\sigma), \tag{1}$$

which is invariant with respect to the substitution $\sigma \to k\sigma$. The components Γ^i_{pq} of the gravitational field are in the respective theories defined by

$$\Gamma_{i,\,pq} = g_{ij}\Gamma^j_{pq} = \frac{1}{2}\left(\frac{\partial g_{ip}}{\partial x_q} + \frac{\partial g_{iq}}{\partial x_p} - \frac{\partial g_{pq}}{\partial x_i}\right), \tag{2E}$$

$$\Gamma_{i,\,pq} = \delta_{ij}\Gamma^j_{pq} = \frac{1}{2}\left(\frac{\partial h_{ip}}{\partial x_q} + \frac{\partial h_{iq}}{\partial x_p}\right) - \frac{\partial h_{pq}}{\partial x_i}, \tag{2B}$$

$$\Gamma_{i,\,pq} = \delta_{ij}\Gamma^j_{pq} = \frac{1}{2}\left(\frac{\partial h_{ip}}{\partial x_q} + \frac{\partial h_{iq}}{\partial x_p} - \frac{\partial h_{pq}}{\partial x_i}\right). \tag{2D}$$

The constant factor γ, specific for the individual particle, is the ratio gravity/mass. Once the cut performed by which D arises from E, a combination $\delta_{ik} + \mu h_{ik}$ with this or that constant μ has as much and as little significance in D as in B. In B and D there is no more reason for γ to be independent of the particle than one can expect the ratio η = charge/mass to be the same for all particles moving in an electromagnetic field according to the equations

$$\frac{d^2x_i}{d\sigma^2} = \eta \cdot F^i_q u^q \qquad \left(F_{ik} = \frac{\partial \varphi_k}{\partial x_i} - \frac{\partial \varphi_i}{\partial x_k}\right).$$

We use the familiar notations

$$ds^2 = g_{ik}dx_i dx_k \text{ in E,}$$

$$ds^2 = \delta_{ik}dx_i dx_k = dx_0^2 - (dx_1^2 + dx_2^2 + dx_3^2) \text{ in B and D.} \quad (3)$$

From (2E) and (2B) there follows the relation

$$\left(\frac{ds}{d\sigma}\right)^2 = \text{const.} \quad (4E, B)$$

Birkhoff's choice of the Γ^i_{pq} was, in fact, suggested by the postulated relation (4B). No such relation (4) holds in D. [One could remedy this by a suitable transformation of the parameter σ before letting $\epsilon \to 0$; the recipe for the construction of D is not entirely unambiguous! But following Mr. Barajas I shall stick to our present interpretation of D.]

What I had in mind when I said that B and D are "essentially of the same nature" (not "much the same," as Mr. Barajas quotes me) were the following points: (1) Both operate in a flat world and are linear with respect to h_{ik}. (2) Both have a *tensor* h_{ik} as gravitational potential, and the ponderomotoric force exerted by the gravitational field depends *quadratically* on u^i. One should not forget that before Einstein a gravitational potential with 10 components was an unheard-of thing; this complication was forced upon him by the principles of general relativity rather than by any observed facts about the dependence of the force of gravitation on velocity. Notice also the formal similarity of the expressions (2B) and (2D). (3) In both theories inertia and gravitation are separate entities; hence no account can be given for the identity of weight and mass, while the equation $\gamma = 1$ is a necessity in E—but pointing to these analogies I did not for a moment suggest that B and D are identical in their numerical consequences!

In a theory of discrete particles the static centrally symmetric solutions of the homogeneous field equations are decisive. With such calibration of the distance $r = (x_1^2 + x_2^2 + x_3^2)^{1/2}$ from the center as renders Einstein's metric 3-space a conformal map of Euclidean space this solution in E is given by

$$ds^2 = \left(\frac{1 - m/2r}{1 + m/2r}\right)^2 \cdot dx_0^2 - (1 + m/2r)^4(dx_1^2 + dx_2^2 + dx_3^2). \quad (5E)$$

It contains only *one* constant m. In the limit for small m/r one obtains

$$h_{00} = -2m/r, \quad h_{0\alpha} = 0, \quad h_{\alpha\beta} = (2m/r)\delta_{\alpha\beta} \quad [\alpha, \beta = 1, 2, 3]. \quad (5')$$

The homogeneous field equations assumed by Birkhoff are

$$\Box h_{ik} = 0 \quad \text{where } \Box\varphi = \delta^{pq}\frac{\partial^2\varphi}{\partial x_p \partial x_q}. \quad (6)$$

Their most general static centrally symmetric solution involves 3 arbitrary constants a, b, l:

$$h_{00} = -2a/r, \qquad h_{0\alpha} = 0;$$
$$h_{\alpha\beta} = (2b/r)\delta_{\alpha\beta} + l(r^{-3}\delta_{\alpha\beta} + 3r^{-5}x_\alpha x_\beta). \qquad (5B)$$

From the present standpoint this is a serious disadvantage of B. (5′) is contained in (5B) as the special case $a = b$, $l = 0$. To have a common basis for the computation of the consequences of (2B) and (2D) I assume the h_{ik} to be given by (5B) with $l = 0$, and set $\gamma = 1$ for all particles.

Then one finds for the period of revolution T of a planet around the sun and for the advance ω of its perihelion in the three competing theories the approximate formulae

$$T^2 \sim 4\pi^2 r_0^3/M, \qquad \omega \sim 2\pi j/r_0(1 - e^2);$$
$$M_E = m, \qquad M_B = 2a, \qquad M_D = a;$$
$$j_E = 3m, \qquad j_B = 2a + 4b, \qquad j_D = 2a + 3b.$$

$r_0(1 - e)$ and $r_0(1 + e)$ are the limits between which the distance of the planet from the center (sun) varies. In carrying out the calculation for D, Mr. Barajas changes at a certain step b into $-b$ and erroneously makes use of the relation (4B) which does not hold in D. In our notations, and with the dot used as an abbreviation of $d/d\sigma$, the correct equations in D read

$$\ddot{x}_0 = -\frac{2a}{r^2}\dot{x}_0\dot{r}$$

$$\ddot{x}_\alpha = -\frac{x_\alpha}{r^3}\{a\dot{x}_0^2 + b(\dot{x}_1^2 + \dot{x}_2^2 + \dot{x}_3^2)\} + \frac{2b}{r^2}\dot{x}_\alpha\dot{r},$$

from which, after proper normalization of the constant factor k in σ and of the invariant plane, follow the integrals:

$$\dot{x}_0 = e^{2a/r},$$
$$x_3 = 0, \qquad x_1\dot{x}_2 - x_2\dot{x}_1 = C\cdot e^{-2b/r},$$

$$\dot{x}_1^2 + \dot{x}_2^2 = \frac{a}{2a + b}(e^{4a/r} - e^{-2b/r}) + C_0\cdot e^{-2b/r}.$$

For the deflection 2δ of a light ray whose asymptote has the distance r_0 from the center one finds

$$\delta \sim i/r_0; \qquad i_E = 2m, \qquad i_B = 2(a + b), \qquad i_D = a + b,$$

if one assumes that the light ray follows a trajectory which satisfies the condition $(ds/d\sigma)^2 = 0$ in E and B and the same condition *at infinity* in D. It is true that the special choice $a = b = m/2$ reduces the *three* values of M, j, i in B to those in E, because the linear relation $M + j = 2i$ satisfied in E holds also in B, whereas $M + j = 3i$ in D.

However, the hypothesis on which our computation of δ is based is of a very dubious nature in B and D. In truth the propagation of light is determined by Maxwell's electromagnetic field equations, from which the concept of light ray emerges by letting the frequency of the light waves tend to infinity. In Einstein's general relativity theory the g_{ik} affect Maxwell's equations in such a way that the light rays thus defined coincide automatically with the nul geodesics; and the deflection $\delta = 2m/r_0$ results. But in B with its flat world there is no necessity for modifying Maxwell's equations, and hence the light rays are straight lines ($i_B = 0$) and do not coincide with the nul trajectories—unless the discrepancy is removed by some artificial modification of the electromagnetic equations which has to be constructed *ad hoc*.

Finally we discuss the relative shift $\Delta\lambda/\lambda$ of wave lengths λ which is observed by a spectroscopist who compares the light emitted from two atoms of the same nature. In Einstein's theory there is no doubt about the principles on which the computation of $\Delta\lambda/\lambda$ rests: the constitution of the atom determines the frequencies of its oscillations measured in terms of its *proper time* s, propagation takes place in spherical waves according to the electromagnetic equations. For the static field (5E), two resting atoms at fixed distances r and a resting observer one thus obtains $\Delta\lambda/\lambda \sim m \cdot \Delta(1/r)$. Desperate steps must be taken to escape the conclusion $\Delta\lambda = 0$ in B. If I do not misunderstand him, Mr. Barajas interprets $dx_0/d\sigma$ as the energy of a photon traveling along its nul trajectory from one to the other atom and, by grafting Planck's quantum law on his classical scheme, assumes that the frequency of the photon varies in proportion to this its energy! In this way (to which the quantum theory of light lends little support) he manages to obtain a red shift $\Delta\lambda/\lambda \sim 2a \cdot \Delta(1/r)$.

It is well known that in E the formula (4E) is nothing but a special case of the law that the scalar product of two vectors v^i, w^i at a common world point P does not change if both undergo parallel displacement to an infinitely near point:

$$dv^i = -\Gamma^i_{pq} v^p dx_q, \qquad dw^i = -\Gamma^i_{pq} w^p dx_q$$

imply $d(v^i w_i) = 0$. Perpendicular vectors remain perpendicular. Among other applications of the latter fact I mention Fokker's computation of the geodesic precession of the Earth's axis,[4] for which, assuming the orbit to be circular, he finds the value $3\pi m/r_0$. How would this phenomenon be treated in B? Birkhoff's rule (2B) naturally fails to produce a law of

constancy of the same generality; and yet the special case where v and w are both parallel to the displacement is made the decisive postulate.

When one passes from discrete particles to continuous distribution of charge, energy and momentum and thus from the homogeneous to non-homogeneous field equations, the divergences between the theories E and B become more pronounced than their analogies, for the simple fact that Birkhoff's theory is based on the duality of field and matter in a form which physics has abandoned during the last 40 years. In his scheme mechanical equations appear independently besides field equations, and matter is represented by a charge vector s^i and an energy-momentum tensor T^{ik} of the type

$$s^i = \rho u^i, \qquad T^{ik} = \mu u^i u^k \tag{7}$$

which involve a velocity field u^i (hydrodynamical models of atoms!). In a steady development physics has come to the conclusion that the mechanical equations, the differential conservation laws for charge, energy and momentum, follow from the field equations, and in formulating its exact field laws it has learned to dispense with quantities like (7) which are tied to the idea of a "fluid," a continuously distributed moving substance. It is true that quantum physics by its statistical interpretation of the field equations gives rise to a certain complementary duality of "wave" and "particle"; but this is an altogether different affair.

A consistent linear field theory of gravitation is well suited to illuminate the problem of energy and momentum; it might also help, though this is much more problematic, in pointing the way to the correct unification of gravitation and electromagnetism. I propose, therefore, to continue this discussion at another place.

[1] Birkhoff, G. D., these PROCEEDINGS, **29**, 231 (1943).

[2] *Mathematical Reviews*, **4**, 285 (1943).

[3] These PROCEEDINGS, **30**, 54 (1944).

[4] *Versl. K. Akad. Wetensch. Amsterdam*, **29**, p. 611; Schouten, J. A., *ibid.*, p. 1150; Weyl, H., *Raum Zeit Materie*, 5th ed., 1923, p. 320.

SCALE CURVES IN GENERAL CARTOGRAPHY*

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1. The square of the linear element dS of a surface Σ is

$$dS^2 = E(x, y)dx^2 + 2F(x, y)dxdy + G(x, y)dy^2, \qquad (1)$$

where $H^2 = EG - F^2 > 0$. Of course (x, y) are general curvilinear coördinates of any point on Σ. Also we may consider (x, y) to represent the cartesian coördinates of any point in a plane π so that the square of the linear element ds on π is $ds^2 = dx^2 + dy^2$. Thus there results a mapping of a surface Σ upon a plane π such that points of Σ and π will correspond if and only if they have the same coördinates. We shall term any particular mapping of a surface Σ upon a plane π a *cartogram*.

We consider the ratio σ of the differentials of the arc lengths on π and Σ, respectively. This scale function σ is defined by the formula

$$\sigma^2 = \left(\frac{ds}{dS}\right)^2 = \frac{1 + y'^2}{E + 2Fy' + Gy'^2} \qquad (2)$$

where the accent denotes differentiation with respect to x.

A scale curve is the locus along which the scale does not vary. There are, in general, ∞^2 scale curves. We shall be chiefly concerned with systems of scale curves, the discussion of which begins in Section 3.

The scale function σ depends upon the lineal-element (x, y, y') only. It is independent of y' if and only if the mapping of Σ upon π is conformal. It will be a mere constant if Σ is a developable surface and the mapping is an unrolling of Σ upon π followed by a similitude on π. Since we have already studied the simple-infinity of scale curves in conformal maps,[1] we shall assume in the present paper that the mapping is not conformal. Then the conditions $F = 0$ and $E = G$ cannot hold simultaneously.

Our new general theory will be applied to classic map projections or cartograms (area-preserving, azimuthal, etc.) in later papers.

2. If on the plane π, the scale function σ is laid off along the corresponding direction y' at a fixed point (x, y), the end-point will describe a locus, which is an ellipse. Let (X, Y) represent the cartesian coördinates of a point on the ellipse relative to the fixed point (x, y), which is, of course, the center of the ellipse. The equation of this terminal ellipse is

$$EX^2 + 2FXY + GY^2 = 1. \qquad (3)$$

The directions of the major and minor axes of this terminal ellipse are given by

$$Fy'^2 + (E - G)y' - F = 0. \tag{4}$$

This defines, according to a theorem of Tissot, the unique orthogonal net of curves on π which by the mapping corresponds to an orthogonal net on Σ. A curve is a characteristic curve of the mapping of Σ upon π if and only if it is a curve of this net.

The semi-major and semi-minor diameters of the terminal ellipse satisfy the equation

$$H^2\sigma^4 - (E + G)\sigma^2 + 1 = 0. \tag{5}$$

For a fixed σ, the preceding equation defines a curve such that at all points of this curve the corresponding terminal ellipses are congruent. This set of $2\,\infty^1$ curves is termed the *singular family* of curves.

(It is noted that this singular family can consist of ∞^1 curves if and only if, either, the mapping is conformal; or else the functions $E + G$ and $H^2 = EG - F^2$ are functionally related. For example, under any area preserving map the singular family is $E + G = \text{const.}$)

3. Upon setting the scale σ of the equation (2) equal to a constant, there result curves along which the scale is constant. *If the mapping is not conformal, there are ∞^2 scale curves.* On the other hand if the mapping is conformal, there are ∞^1 scale curves. Obviously, all curves are scale curves if Σ is a developable surface and the mapping is an unrolling of Σ upon π followed by a similitude on π.

Henceforth only the general case where the mapping is not conformal shall be considered. It is observed that for a fixed σ, there pass two scale curves through any point of the plane. However, at any point of the curve (5), the two scale curves will be tangent. This supplies us with the reason for calling the family (5) singular.

Upon eliminating the constant σ from the equation (2) by differentiation, we discover that *the differential equation of our ∞^2 scale curves is*

$$y'' = \frac{(1 + y'^2)[E_x + y'(E_y + 2F_x) + y'^2(2F_y + G_x) + y'^3G_y]}{2[-F + y'(E - G) + y'^2F]} \tag{6}$$

It is seen that not every system of ∞^2 curves can represent the scales of a general mapping of a surface Σ upon a plane π, because (6) is of special algebraic form in the first derivative.

We observe from (6) that there are *three inflectional directions and two cuspidal directions for the scale curves. The cuspidal directions are always orthogonal.*

4. Let (X, Y) represent the cartesian coördinates relative to the point (x, y) of the center of curvature constructed at the point (x, y) of a scale curve. For a fixed point (x, y), the corresponding centers of curvature (the central locus) of the ∞^1 scale curves through the fixed point describe the cubic curve

$$G_y X^3 - (2F_y + G_x)X^2 Y + (E_y + 2F_x)XY^2 - E_x Y^3 + 2[-FX^2 + (E - G)XY + FY^2] = 0. \quad (7)$$

The locus of the centers of curvature (the central locus) is a general cubic curve which has a node at the fixed point (x, y), the directions of the tangent lines at the node coinciding with the characteristic directions.

This property is possessed not only by any family of ∞^2 scale curves but also by a more general class of families of curves. If a system of curves possesses the preceding property, then it must be defined by a differential equation of the form

$$y'' = \frac{(1 + y'^2)(\alpha + \beta y' + \gamma y'^2 + \delta y'^3)}{2(-\eta + \epsilon y' + \eta y'^2)}. \quad (8)$$

This family will represent scale curves if and only if the six functions $(\alpha, \beta, \gamma, \delta, \epsilon, \eta)$ of (x, y) satisfy the two partial differential equations of second order

$$M_y = N_x,$$
$$\alpha_y + N\alpha = \delta_x + \epsilon_{xy} + N\epsilon_y + \epsilon N_y + M(\delta + \epsilon_y + \epsilon N), \quad (9)$$

where M and N are defined by

$$(\epsilon^2 + 4\eta^2)M = \epsilon(\alpha - \gamma) + 2\eta(\beta - \delta) - 4\eta\eta_x + 2\epsilon\eta_y - \epsilon\epsilon_x - 2\eta\epsilon_y,$$
$$(\epsilon^2 + 4\eta^2)N = -2\eta(\alpha - \gamma) + \epsilon(\beta - \delta) - 2\epsilon\eta_x - 4\eta\eta_y + 2\eta\epsilon_x - \epsilon\epsilon_y. \quad (10)$$

There are essentially ∞^2 surfaces which possess the same family of ∞^2 scale curves. If the surface Σ with the linear element (1) is mapped upon the plane π such that the differential equation (6) represents the scale curves, then the linear element dS' of any surface Σ' with the same scale curves must be given by

$$dS'^2 = adS^2 + bds^2 \quad (11)$$

where $a \neq 0$ and b are constants.

It must be emphasized that the results of this section are valid only for the general case where the cubic curve (7) is not degenerate.

The class of scale curves is converted into itself only by the conformal group. This is true also for the class of curves defined by differential equations of the type (8).

5. If the parametric curves of Σ are not orthogonal, that is, $F \neq 0$, the cubic curve (7) may degenerate into three straight lines, two of which are the tangent lines of the characteristic curves at the fixed point, and the other is the general line

$$G_y X + E_x Y - 2F = 0. \quad (12)$$

In this event, the ∞^2 scale curves form a velocity system.[2] The necessary and sufficient conditions that the ∞^2 scale curves (6) form a velocity family are

$$\begin{aligned} G_y(E - G) &= F(E_x + G_x + 2F_y), \\ -E_x(E - G) &= F(E_y + G_y + 2F_x). \end{aligned} \tag{13}$$

The differential equation of the ∞^2 scale curves is then

$$y'' = (1/F)(1 + y'^2)(-E_x + y'G_y). \tag{14}$$

If the scale curves form a natural family, then the characteristic curves are an isothermal net. The curves $(E - G)^2 + 4F^2 = \text{const.}$ form an isothermal family if the scale family is isogonal. The scale family is a conformal rectilinear wex if and only if the function $\log [E - G - 2iF]$ is an analytic function of $z = x + iy$ only. Therefore if the scale family is a conformal rectilinear wex, the curves $E - G = \text{const.}$ and $F = \text{const.}$ form an isothermal net.

A system of ∞^2 scale curves can be of the cubic (Lie-Liouville) type[3] *if and only if it is a velocity system.*

6. If the parametric curves are orthogonal, so that $F = 0$, the cubic curve (7) can be degenerate if and only if $G_y = 0$ or $E_x = 0$. In this event, the cubic curve consists of the tangent line to one of the characteristic curves and a conic section tangent to the other characteristic curve at the given point. This may also happen in the case where the parametric curves are not orthogonal.

If $G_y = E_x = 0$, together with the condition $F = 0$, the ∞^2 scale curves form the velocity system

$$y'' = \frac{1}{E - G}(1 + y'^2)(E_y + G_x y'). \tag{15}$$

In this case, the cubical central locus consists of the tangent lines of the characteristic curves at the fixed point, and the line

$$-G_x X + E_y Y + 2(E - G) = 0. \tag{16}$$

7. Finally we determine the class of surfaces Σ for which there exists a map of Σ upon the plane π such that the scale curves coincide with the totality of α^2 straight lines. This class of surfaces Σ is given by

$$\begin{aligned} dS^2 = a_0(ydx - xdy)^2 + 2(a_1dx + b_1dy)(ydx - xdy) + a_2dx^2 \\ + 2c_2dxdy + b_2dy^2. \end{aligned} \tag{17}$$

For this class of surfaces, we find

$$\begin{aligned} H^2 = (a_0a_2 - a_1^2)x^2 + 2(a_0c_2 - a_1b_1)xy + (a_0b_2 - b_1^2)y^2 + 2(a_1c_2 - a_2b_1)x \\ + 2(a_1b_2 - b_1c_2)y + (a_2b_2 - c_2^2). \end{aligned} \tag{18}$$

The gaussian curvature G is given by

$$H^4G = -2a_0H^2 + a_0(c_2^2 - a_2b_2) + a_2b_1^2 - 2a_1b_1c_2 + a_1^2b_2. \quad (19)$$

Thus the guassian curvature G is found to be a rational function of (x, y).

In the general case (17), it is found that the ∞^1 straight scales corresponding to a given scale are tangent to a conic section. By varying σ we find that the conic sections form a confocal family. It is a central conical or parabolic family according as $a_0 \neq 0$ or $a_0 = 0$.

If the gaussian curvature G is identically constant, then G must be identically zero. In this case $a_0 = a_1 = b_1 = 0$. This means that if the surface Σ is of constant curvature such that the scale curves are straight lines, then Σ must be developable, and the mapping of Σ upon π is a development of Σ upon π followed by an affine transformation. To a given scale σ, corresponds a parallel pencil of straight lines.

Thus there exists no non-conformal map of a sphere upon a plane such that the scale curves coincide with the totality of ∞^2 straight lines. From this, we deduce that the only map (conformal or not) of a sphere upon plane such that the scale curves are straight lines is the Mercator projection (in which case we have a single-infinitude of parallel straight lines as scale curves).

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[1] See the paper, "Scale Curves in Conformal Maps," *Proc. Nat. Acad. Sci.*, (1944). Also see "Scale Curves in Cartography," *Science*, **98**, 324–325 (1943), and *Science News Letter*, March 25 (1944).

[2] A general velocity system is defined by the differential equation $y'' = (1 + y'^2)(\psi - y'\phi)$ where ϕ and ψ are functions of (x, y). See Kasner, "Differential Geometric Aspects of Dynamics," *Amer. Math. Soc. Colloquium Publications*, **3** (1912, 1934); *Trans. Amer. Math. Soc.*, **9, 10** (1908–1909). See also Kasner and De Cicco, "The Geometry of Velocity Systems," *Bull. Amer. Math. Soc.*, **49**, 236–245 (1943), and Kasner, "Geometric Properties of Isothermal Families," *Instituto de Matematica*, Rosario, Argentina, 5 (1943), volume in honor of Rey Pastor.

[3] A differential equation of the cubic type is of the form $y'' = \alpha + \beta y' + \gamma y'^2 + \delta y'^3$, where $(\alpha, \beta, \gamma, \delta)$ are functions of (x, y) only. This has been studied extensively by Lie, R. Liouville, Tresse, Kasner and Wilczynski. With respect to the group of arbitrary point transformations, this type is called a differential equation of the first rank. See Kasner, "The Geometry of Differential Elements of the Second Order with Respect to the Group of All Point Transformations," *Amer. Jour. Math.*, **28**, 203–213(1907).